speckledness of the hen? It cannot simply take an average of some visual property of the scene (as is the case with orientation [14]) without first normalising the size and shape of each speckle. Second, which brain system implements this mechanism? Burr and Ross [3] cite evidence that the intraparietal sulcus responds to the number of objects in a display [15] even when the total continuous extent of the objects is taken into account [16]. But the intraparietal sulcus represents numerosity quite abstractly: independently of whether the objects are distributed in space or in time [16] and independently of modality [15]. Because the adaptation phenomenon described here is retinotopic, earlier stages in neural visual processing are implicated as well.

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Cell–Matrix Adhesion: The Wech Connection

Integrins link the extracellular matrix to the cytoskeleton via a complex of proteins: the integrin–cytoskeleton link. A recent study in *Drosophila* has uncovered a new component of the link, Wech, and shown that it is essential for integrin-mediated adhesion.

Isabelle Delon and Nick Brown

Cell adhesion in multicellular organisms relies on highly conserved multi-protein complexes. Attachment of cell layers to each other is mediated by the integrin family of transmembrane receptors. Integrins connect to ligands in the extracellular matrix, and to the cytoskeleton inside the cell [1]. This connection is the basis of cellular junctions that mediate stable adhesion in tissues. Integrins are also essential for cell migration over the extracellular matrix. The assembly of the organism requires integrins to mediate attachment between cell layers, such as the attachment of the dermis to the epidermis in mice [2], or of muscles to the body wall in worms and flies [3]. Disrupting integrin function results in separation of these cell layers and impairment of migration, and the subsequent death of the animal. Integrins do not attach to the cytoskeleton directly, but via a complex of proteins, or the 'integrin–cytoskeleton link' (the link) [4]. Disrupting the function of one of these components can be as deleterious as disrupting integrins themselves, stressing their significance for integrin-mediated adhesion. A recent paper from Löer *et al.* [5] reports the identification of a new essential member of the integrin–cytoskeleton link.

The molecular composition of this link has been extensively studied in many systems, and 156 components have been collated so far that may contribute to it [6]. The multi-protein complex identified was called 'the adhesome', and includes the link as well as proteins involved upstream and downstream. Amongst the components of the adhesome are 90 'intrinsic' components which physically localise to adhesion sites, and 66 'peripheral' components affecting the activity of the intrinsic ones. Four functional families of adhesome components can be defined: adhesion receptors, adaptors and actin regulators, which form the physical structure of the adhesion site; and signalling molecules, consisting mostly of enzymes that modify the interactions and signal inside the cell. Löer *et al.* [5] report that mutation of the *Drosophila wech* gene mimics the absence of integrins in the embryonic muscles. The Wech protein is concentrated at sites of integrin adhesion, such as the muscle ends, and require talin to be positioned there. In absence of Wech, integrin-linked kinase (ILK) and tensin are reduced, but PINCH is still localised (Figure 1). These data suggest that Wech provides a link between talin and ILK, and this was confirmed by finding that Wech binds to both proteins. Mutation of the *wech* gene causes a stronger phenotype than that of *ilk,* suggesting that Wech does more than just recruiting ILK. From these data Wech can be classified as an adaptor molecule.

Given that so many proteins have been implicated as adhesome components already, why is it remarkable to find a new one? First of all, Wech is a member of a protein family that contains domains not so far documented in the 156 other known adhesome proteins. Second, the other members of the Wech family have very different functions, such as regulating cell proliferation and tumour suppression. Third, it is exciting that forward genetic

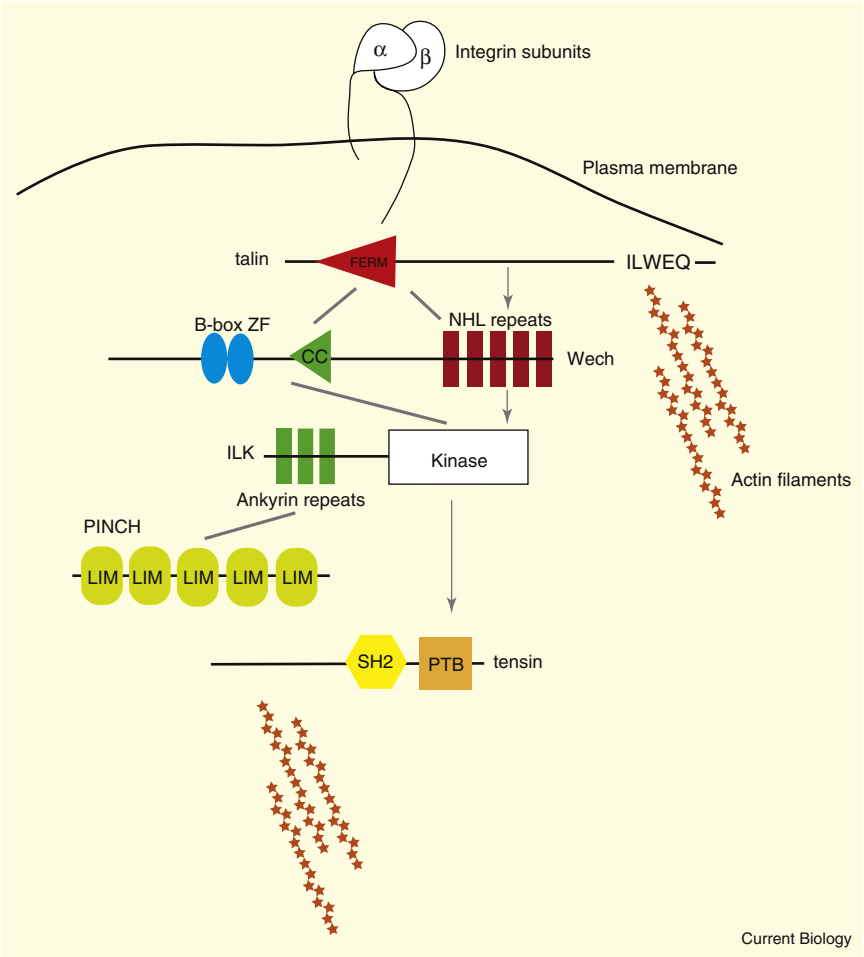

Figure 1. Architecture of the integrin–cytoskeleton link in *Drosophila*.

Schematic representation of the proteins involved in integrin-mediated adhesion. Integrins are transmembrane receptors that connect the cell to the extracellular matrix. The cytoplasmic tail of the integrin beta-subunit binds talin and thus mediates the link to the intracellular protein complex. Binding is shown by grey lines, and the recruitment hierarchy by grey arrows. Wech interacts via its B-box coiled coil region (BBC) and its NHL repeats with the head domain of talin comprising the FERM domain, and via its BBC region to the kinase domain of ILK. PINCH binds to ILK, but PINCH recruitment is neither ILK- nor Wech-dependent. Talin and tensin can link actin filaments. According to the adhesome classification, talin and tensin are thus actin regulators, whereas the other molecules represented are adaptors. It is noteworthy that such actin regulators have different places in the hierarchy of recruitment, suggesting that the actin link is made at different levels.

approaches in model organisms can still add new conserved components to a very well studied complex.

The most common domains in proteins of the link identified so far include LIM, SH2, SH3, CH, PTB and FERM domains [7]. These are all well-identified protein–protein interaction domains. Wech contains a B-box zinc finger, a coiled-coil domain and NHL (NCL-1, HT2A, LIN-41) repeats. Although each domain individually is widely represented across species, this combination of domains is found in only four proteins in *Drosophila*. The mammalian counterpart of Wech also contains a RING domain, and belongs to the family of tripartite motif proteins RBCC (Ring-B-box-Coiled-Coil), containing or not NHL repeats [8]. The B-box motif is found primarily in transcription factors, ribonucleoproteins and proto-oncoproteins, but no clear function has been assigned to it. NHL repeats are stretches of about 40 amino acids, and both functional studies and the wide occurrence of the domain in very diverse proteins suggests that it is involved in protein–protein interaction [8–10]. The other three *Drosophila* proteins containing a B-box and NHL repeats are the tumour suppressor brain tumour (Brat), the meiotic protein Mei-P26 and a newly identified muscle-specific protein Another B-Box Affiliate (ABBA) [11] (Figure 2).

Depletion of either Brat or Mei-P26 induces a tumorous phenotype in *Drosophila*. Brat was identified as a growth inhibitor that is asymmetrically segregated during nervous system morphogenesis. Larval neuroblasts, which are the stem cells of the adult brain, normally divide into a ganglion mother cell (GMC) and a neuroblast. In the absence of Brat, both daughter cells stay as neuroblasts and thus undergo too many divisions, leading to brain tumours. Brat is a regulator of dMyc, affecting protein translation [12]. Inactivation of *mei-P26* leads to overproliferation of germline stem cells. It genetically interacts with *bag of marbles*, an inhibitor of proliferation in daughter cells, suggesting that the ovarian tumours in *mei-P26* mutants are caused by a lack of proliferation control in the stem cells [13]. Moreover, mutations truncating the NHL repeats of Brat result in neoplastic larval brains, indicating that the tumour suppressor function of Brat requires the NHL repeats [14]. It was therefore suggested that NHL repeats could have general oncogenic properties by regulating proliferation in stem cells [12].

Could the phenotype of *wech* mutants be caused by a similar oncogenic defect? Löer *et al.* [5] have ruled out the possibility that the integrin-like muscle detachment phenotype caused by the absence of *wech* is due to overproliferation, indicating a different mode of activity for Wech in muscles. Wech and Brat share the characteristic that they are tightly localised in protein complexes within the cell, and this may be a key feature of their activity. Finally, although the concentration of ILK with integrins is greatly reduced in *wech* mutant, it is not totally abolished, suggesting some redundancy in the mechanism to recruit ILK. It is not known whether ABBA has any integrin-related function for muscle attachment, and it will be of interest to test the possible redundancy between the related proteins Wech and ABBA.

The outstanding finding of the Löer *et al.* [5] paper is the discovery of a new member of the integrin–cytoskeleton

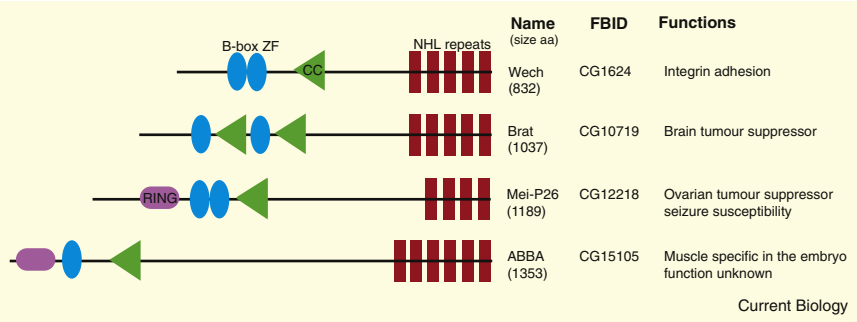

Figure 2. B-Box NHL domain proteins in *Drosophila*.
The four proteins containing B-box and NLH domains encoded by *Drosophila* genes are represented. The Flybase ID (FBID), size of the protein and known functions are indicated.

link that is evolutionarily conserved. The authors show that the mouse homologue of Wech also localises to integrin junctions with ILK, consistent with a conserved function in integrin-mediated adhesion. Like Wech, many components of the integrin–cytoskeleton link known to date in flies have been identified in genetic screens. Prior screens have focused on searching for the more easily visualised integrin loss-of-function phenotype, the wing blister [15]. The wing is a double layer of epithelial cells with the basal surface of the two layers attached through the extracellular matrix. In the absence of integrins or components of the link, the layers separate and form a blister [16]. Talin, PINCH and tensin were identified in this way, whereas *ilk* mutants were generated by reverse genetic approaches [7]. Wech, however, was found looking for a different integrin phenotype, the detachment of the embryonic muscles from the epidermis. The success of this novel approach suggests that many new components may be found by screening for mutants that give tissue specific integrin-like phenotypes. Results to date have shown that whereas tensin is only required in the wing [17], no component has been identified so far that just affects muscles. It will be thus exciting to know whether Wech affects other integrin mechanisms as well. Our idea of the mechanism of action of the integrin–cytoskeleton link is that the more integrin functions that are affected when a particular component is inactivated, the more 'core' this member is to the complex. This view might be challenged, however, by the discovery of truly tissue-specific components.

An intriguing feature of the integrin–cytoskeleton link in *Drosophila* is that, while most components are found at all sites of integrin function, several only cause defects in some tissues when inactivated. This is, for example, the case for tensin and focal adhesion kinase (FAK), which affect only the wing and the olfactory stalk, respectively [17–19]. Even more curious is the example of vinculin, a well-established member of the link in vertebrate cells in culture that has no apparent function in flies [20]. A hypothesis to explain this aberration is that the components of the link form a toolkit that is differentially used in various developmental situations, or that some components are present in a 'ready to strike' state in the event of a critical developmental or environmental accident. Supporting this is the predominance of phosphorylation cascades happening in response to adhesion, that can quickly change the interactions inside the complex [6]. Furthermore, the toolkit hypothesis implies that the molecular composition, or usage, of the integrin–cytoskeleton link could modulate the properties of the adhesive junction, making it weaker or stronger. The identification of new types of proteins involved in the link, like Wech, will help to discover the many functions of this toolkit.

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